

That which is claimed is:

1. A method for modulating the expression of an exogenous gene in a mammalian subject containing:

5 (i) a DNA construct comprising said exogenous gene under the control of an ecdysone response element; and

10 (ii) a modified ecdysone receptor which, in the presence of a ligand therefor, and optionally in the further presence of a receptor capable of acting as a silent partner therefor, binds to said ecdysone response element;

15 said method comprising administering to said subject an effective amount of a ligand for said modified ecdysone receptor; wherein said ligand is not normally present in the cells of said subject; and wherein said ligand is not toxic to said subject.

2. A method according to claim 1 wherein said modified ecdysone receptor comprises:

a ligand binding domain capable of binding an ecdysteroid;

5 a DNA-binding domain obtained from a DNA-binding protein; and

an activation domain of a transcription factor,

10 wherein at least one of said DNA-binding domain or said activation domain is not obtained from a native ecdysone receptor,

with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an  
15 *E. coli* LexA protein.

09042438-031698

sub B1  
SUB  
D1

3. A method according to claim 2 wherein said modified ecdysone receptor is further characterized as having substantially no constitutive activity in mammalian cells.

4. A method according to claim 2 wherein the DNA-binding domain of said modified ecdysone receptor is derived from a member of the steroid/thyroid hormone superfamily of receptors.

5. A method according to claim 2 wherein said activation domain is obtained from a member of the steroid/thyroid hormone superfamily of receptors.

6. A method according to claim 2 wherein said activation domain is selected from a glucocorticoid receptor activation domain, a VP16 activation domain or a GAL4 activation domain.

7. A method according to claim 6 wherein said modified ecdysone receptor is selected from VpEcR, VgEcR or GEcR.

8. A method according to claim 7 wherein said modified ecdysone receptor is VgEcR having the amino acid sequence set forth in SEQ ID NO:5.

9. A method according to claim 1 wherein said modified ecdysone receptor is present primarily in the form of a homodimer.

10. A method according to claim 9 wherein said ecdysone response element is the native ecdysone response element.

Sub  
Fa

090443-031698  
SUB  
ID

Sub  
h1

11. A method according to claim 1 wherein said receptor capable of acting as a silent partner is RXR.

12. A method according to claim 11 wherein said RXR is exogenous to said mammalian subject.

13. A method according to claim 1 wherein said ecdysone response element is a modified response element which comprises, in any order, a first half-site and a second half-site separated by a spacer of 0-5 nucleotides;  
 5 wherein said first half-site has the sequence:

-RGBNNM-,

wherein

each R is independently selected from A or G;  
 10 each B is independently selected from G, C, or T;  
 each N is independently selected from A, T, C, or

G; and

each M is independently selected from A or C;  
 with the proviso that at least 4 nucleotides of each  
 15 -RGBNNM- group of nucleotides are identical with the  
 nucleotides at comparable positions of the sequence  
 -AGGTCA-; and

said second half-site is obtained from a glucocorticoid receptor subfamily response element.

14. A method according to claim 13 wherein said response element has substantially no binding affinity for farnesoid X receptor (FXR).

15. A method according to claim 1 wherein said ligand is a naturally occurring ecdysone, an ecdysone-analog or an ecdysone mimic.

16. A method according to claim 15 wherein said naturally occurring ecdysone is  $\alpha$ -ecdysone or  $\beta$ -ecdysone.

Sub  
102  
Sub  
h1

Sub  
DB  
031698

Sub  
h1

17. A method according to claim 15 wherein said ecdysone analog is ponasterone A, ponasterone B, ponasterone C, 26-iodoponasterone A, muristerone A, inokosterone or 26-mesylinokosterone.

18. A method according to claim 15 wherein said ecdysone mimic is 3,5-di-tert-butyl-4-hydroxy-N-isobutylbenzamide, 8-O-acetylharpagide, a 1,2-diacyl hydrazine, an N'-substituted-N,N'-disubstituted hydrazine, a 5 dibenzoylalkyl cyanohydrazine, an N-substituted-N-alkyl-N,N-diaroyl hydrazine, an N-substituted-N-acyl-N-alkyl, carbonyl hydrazine or an N-aroyl-N'-alkyl-N'-aroyl hydrazine.

19. A method according to claim 1 wherein said exogenous gene is a wild type gene and/or therapeutic gene.

20. A method according to claim 19 wherein said wild type gene is selected from genes which encode products:

the substantial absence of which leads to the occurrence of a non-normal state in said subject; or

a substantial excess of which leads to the occurrence of a non-normal state in said subject.

21. A method according to claim 19 wherein said therapeutic gene is selected from those which encode products:

which are toxic to the cells in which they are expressed; or

which impart a beneficial property to said subject.

SUB  
h1

09042488-03168

SUB F4

SUB  
D4

22. A method of inducing the expression of an exogenous gene in a mammalian subject containing:

- 5 (i) a DNA construct comprising an exogenous gene under the control of an ecdysone response element,
- 10 (ii) DNA encoding a modified ecdysone receptor under the control of an inducible promoter; wherein said modified ecdysone receptor, in the presence of a ligand therefor, and optionally in the further presence of a receptor capable of acting as a silent partner therefor, binds to said ecdysone response element, and
- 15 (iii) a ligand for said modified ecdysone receptor;

said method comprising subjecting said subject to conditions suitable to induce expression of said modified ecdysone receptor.

20

23. A method of inducing expression of an exogenous gene in a mammalian subject containing a DNA construct containing said exogenous gene under the control of an ecdysone response element, said method comprising

5 introducing into said subject:

- a modified ecdysone receptor; and
- a ligand for said modified ecdysone receptor,
- wherein said receptor, in combination with a
- 10 ligand therefor, and optionally in the further presence of a receptor capable of acting as a silent partner therefor, binds to said ecdysone response element, activating transcription therefrom.

SUB  
D4  
CONT.  
"031598"

24. A method for the expression of a recombinant product detrimental to a host organism, said method comprising:

transforming suitable host cells with:

- 5 (i) a DNA construct encoding said recombinant product under the control of an ecdysone response element, and
- (ii) DNA encoding a modified ecdysone receptor;
- 10 growing said host cells in suitable media; and inducing expression of said recombinant product by introducing into said host cells ligand(s) for said modified ecdysone receptor, and optionally a receptor capable of acting as a silent partner for said modified
- 15 ecdysone receptor.

25. A pharmaceutically acceptable formulation comprising at least one ecdysteroid and a pharmaceutically acceptable carrier.

26. A formulation according to claim 25 wherein said pharmaceutically acceptable carrier renders said formulation suitable for oral, topical, nasal, transdermal, intravenous, subcutaneous, intramuscular, intracutaneous,

5 intraperitoneal or intravascular administration.

27. A formulation according to claim 25 wherein said ecdysteroid is a naturally occurring ecdysone, an ecdysone-analog or an ecdysone mimic.

28. A formulation according to claim 27 wherein said naturally occurring ecdysone is  $\alpha$ -ecdysone or  $\beta$ -ecdysone.

SUB  
D4  
CONT.

09042453 031698

Sum  
a 7

30. A formulation according to claim 27 wherein said ecdysone mimic is 3,5-di-tert-butyl-4-hydroxy-N-isobutyl-benzamide, 8-O-acetylharpagide, a 1,2-diacyl hydrazine, an N'-substituted-N,N'-disubstituted 5 hydrazine, a dibenzoylalkyl cyanohydrazine, an N-substituted-N-alkyl-N,N'-diaroyl hydrazine, an N-substituted-N-acyl-N-alkyl, carbonyl hydrazine or an N-aroyle-N'-alkyl-N'-aroyle hydrazine.

31. A pharmaceutically acceptable formulation consisting essentially of at least one ecdysteroid and a pharmaceutically acceptable carrier.

32. A formulation according to claim 31 wherein said ecdysteroid is a naturally occurring ecdysone, an ecdysone-analog or an ecdysone mimic.

33. A kit comprising at least one ecdysteroid and a pharmaceutically acceptable carrier therefor.

~~34. A formulation according to claim 33 wherein  
said ecdysteroid is a naturally occurring ecdysone, an  
ecdysone-analog or an ecdysone mimic.~~

add B4